Docket No. 15672US01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:	)
C. Randal Mills *et al.*	)
Serial No.: 10/828,653	)
Filed: April 20, 2004	)
For: Process and Apparatus for Treating Implants Comprising Soft Tissue	)
Examiner: Regina M. Yoo	)
Group Art Unit: 1797	)

DECLARATION UNDER 37 CFR 1.132

1. My name is Arunas A. Zhukauskas, and I am over 18 years of age. I am a citizen of the United States of America, and I reside at 14131 NW 25th Avenue, Gainesville, Florida.

2. I understand that this declaration is being submitted to establish the patentability of the claims in the application identified above.

3. Since 1998, I have worked at RTI Biologics, Inc. (previously Regeneration Technologies, Inc.). I have worked in the field of tissue processing for 10 years. When I refer to "tissue processing," I mean the treatment of autograft, allograft, or xenograft tissue to make it more suitable for implantation in a recipient.

4. Application No. 10/828,653 is owned by my employer RTI Biologics, Inc. ("RTI"). I am paid a salary and have stock options in RTI, and I am a Senior Engineer in

the Sports Medicine business unit, and that application relates to technology within my business unit. I know that I am required to testify truthfully in this declaration, as reflected by the last paragraph before my signature.

5. My group at RTI recently conducted a study to evaluate the effect of applying tension to soft tissue implants during a sterilization process. In order to confirm that tensioning during the sterilization process would have an effect on tendon strength after the sterilization process, an assortment of tendons were tested from various donors. Table 1 provides a summary of the tendons that were tested.

Table 1: Logistics of Human Tendons Tested

Tendon Type	Donor #	Age	Gender
Posterior Tibialis	52720	44	M
Achilles	53403	20	F
Peroneous Longus	48726	44	M
Anterior Tibialis	48359	25	F
Anterior Tibialis	50341	51	M
Posterior Tibialis	50341	51	M
Peroneous Longus	49928	59	M

For each pair of tendons (listed above) obtained from a single donor, one tendon of the pair was subjected to a sterilization process while tension was applied. Tension was applied to the tendons by placing the tendons on tensioners similar to those shown in Figure 4 of the present application. The tensioned tendons were then placed in the chamber used for the sterilization process, and they remain tensioned throughout the sterilization process. The contralateral tendon (the other tendon of the pair from the

opposite leg of the same donor) also underwent the same sterilization process at the same time, except that no tensioning was applied to the contralateral tendon during the sterilization process. Therefore, the study design allowed a paired comparison between tendons from the same donor to be performed.

6. The tensioned and non-tensioned tendons were subjected to the same sterilization process. The sterilization process included the steps of contacting the implant with a protective agent selected from the group consisting of alcohols and polyols; contacting the implant with an oxidizing sterilant; and contacting the implant with a rinsing fluid. The sterilization process included other steps as well.

7. After the sterilization process, all tendons from the study were tested on a material testing machine in a tensile manner until failure in order to determine the strength of each sample. More particularly, a length of not less than 75mm was selected from the proximal end of each tendon for testing, and the tendons were cut to the desired length. For each donor matched contralateral tendon pair, the length was nominally the same between the tensioned and non-tensioned sample. The proximal segment of the tendon was then clamped onto a hydraulically driven MTS 858 Bionix Servo-Hydraulic Materials Testing Machine with an attached 25 kilonewton load cell.

8. Each tendon was subjected to the same test protocol, which included the application of a pre-defined and automated load profile, culminating in a pull to ultimate failure load for that tendon. In order to effectively clamp tendons and prevent slipping during testing, upper and lower grips were chilled using dry ice around each clamp. A heated water jacket was employed to keep the gage length of the tendon (the part that

is subjected to tensile strength testing) at a temperature of approximately 37°C.

9. Tables 3 and 4 list the relative pull strengths that were measured for each tendon in this study. Ultimate Tensile Force is the absolute amount of force causing the tendon to fail, or rupture. Ultimate Tensile Stress is the same measurement normalized for the cross-sectional area of the gage length of the tendon.

Table 3: Ultimate Tensile Force (UTF) per sterilized tendon

Tendon Type	Donor #	*T (N)	*NT (N)
Posterior Tibialis	52720	2618	878
Achilles	53403	5889	3119
Peroneous Longus	48726	3298	1636
Anterior Tibialis	48359	2645	1810
Anterior Tibialis	50341	3537	2862
Posterior Tibialis	50341	2881	2499
Peroneous Longus	49928	3370	1370

* T=Tensioned, NT=Not/non-Tensioned, N=Newtons

Table 4: Ultimate Tensile Stress (UTS) per sterilized Tendon

Tendon Type	Donor #	*T (MPa)	*NT (MPa)
Posterior Tibialis	52720	119.6	21.12
Achilles	53403	83.98	56.94
Peroneous Longus	48726	109.3	48.85
Anterior Tibialis	48359	141.0	90.12
Anterior Tibialis	50341	166.9	137.3
Posterior Tibialis	50341	192.9	165.4
Peroneous Longus	49928	119.6	21.11

* T=Tensioned, NT=Not/non-Tensioned, MPa=megapascals

10. These results show a clinically relevant difference between tensioned, sterilized tendons and the non-tensioned, sterilized tendons, with respect to the properties of ultimate tensile force and ultimate tensile strength. This indicates that applying tension to the tendons during the sterilization process yield tendons having improved strength as compared to non-tensioned tendons, in that more tensile force was required to cause the tendons to fail. The non-tensioned tendons failed more easily (with less force applied) than the tensioned tendons.

11. From the data in Tables 3 and 4, we conducted the following statistical analysis using MiniTab version 15. "StDev" stands for standard deviation. "SE Mean" stands for standard error in the mean. "CI" stands for confidence interval. Table 5 shows the comparison of ultimate tensile force (UTF) between tensioned (T) and non-tensioned (NT) groups.

Table 5: Paired T-Test Comparison of UTF and CI: T, NT

	N	Mean	StDev	SE Mean
Tensioned tendons	7	3463	1129	427
Non-tensioned tendons	7	2025	823	311
Difference	7	1438	846	320

95% CI for mean difference: (655, 2220)
T-Test of mean difference = 0 (vs not = 0): T-Value = 4.50 **P-Value = 0.004**

The same comparison was performed for the ultimate tensile stress (UTS) for each of the groups (T and NT) described above, and is shown in Table 6.

Table 6: Paired T-Test Comparison of UTS and CI: T, NT

	N	Mean	StDev	SE Mean
Tensioned tendons	7	133.3	36.8	13.9
Non-tensioned tendons	7	77.3	56.4	21.3
Difference	7	56.1	31.6	12.0

95% CI for mean difference: (26.8, 85.3)
T-Test of mean difference = 0 (vs not = 0): T-Value = 4.69 **P-Value = 0.003**

12. As shown by the statistical analysis, there is a statistically significant difference between the tensioned, sterilized tendons and the non-tensioned, sterilized tendons, with respect to ultimate tensile force and ultimate tensile strength. On average, the tendons that were not tensioned during the sterilization process were 1438 N weaker (p-value= 0.004) than the tendons that were tensioned. These tendons also showed a decrease of 56.05 MPa, on average (p-value=0.003), compared to tensioned tendons.

13. The number of samples tested in this study were sufficient to resolve potential differences between these two groups on the order of 1251 N in force and 46.73 MPa of stress, thus indicating that the study was sufficiently powered to detect the differences that were observed.

14. I make this declaration having been warned that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. 1001) and may

jeopardize the validity of the application or any patent issuing thereon. All statements made of my own knowledge are true and that all statements made on information and belief are believed to be true.

Respectfully submitted,

Date: 04/18/2008

Arunas A. Zhukauskas